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SOIL ALGAL RELATIONSHIPS TO *ONYCHIURUS FOLSOMI*, A MINUTE ARTHROPOD

Linda-Lee McGurk

Department of Botany
University of Hawaii
Honolulu, Hawaii 96822

ISLAND ECOSYSTEMS IRP

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PREFACE

The following text is essentially the master's thesis of Linda Lee McGurk who was, while a graduate student, the principal research assistant on the Island Ecosystems IRP algal project. The personal time invested in technique development and in carrying out this work hardly shows adequately in this finished report.

This work naturally fits into the overall algal project of the Island Ecosystems IRP. There is very little known of tropical terrestrial algae and thus a purely taxonomic effort had to be made in order to record the abundance of the algal species. This was done by sampling both at different elevations, and at similar sites at the same elevations in different seasons. Both sampling schemes were concerned with such objectives as learning about speciation, diversity in the algal communities, community variation as a function of spatial variations in environment, then also as a function of succession and, finally, the roles of the algae in the environments where they are found. The present thesis is an initial venture into this latter area of endeavor.

Maxwell S. Doty

Principal Investigator
Algal portion
US IBP/ISLAND ECOSYSTEMS IRP

ABSTRACT

Elucidation of the roles of the soil algae is a natural goal of the algal component study of the Island Ecosystems IRP. Thus, the present study investigated the possibility that the algae could serve as food for the 5 to 6 mm long insect, Onychiurus folsomii, the most ubiquitous of the soil arthropods in the Hawaii Volcanoes National Park. This goal was sought through a series of laboratory experiments. In some algae grown on Bold's basal medium solidified with agar it was found that 15 species of algae in unialgal culture were ingested. Some of the algal species grew after transiting the insects and perhaps their progeny were recycled. While no algae were seen in the gut of any wild insect, the algae and insects occur together. It was found, however, that in the laboratory the insects grew and reproduced more when algae were present than when on agar medium alone. Using carbon-14 marked algal cells it was demonstrated that labeled material from the algae passed out into the insect tissues increasingly with time as the cells passed through insect gut. It is concluded such soil insects as Onychiurus can grow and reproduce on algae found in their environment and may do so in nature.

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INTRODUCTION

There is little information on the roles of algae in the soil environment. Most of the work on soil algae has been taxonomic (e.g., Groover and Bold, 1969) or qualitative (e.g., Deason and Bold, 1960) and until recently almost nothing was known (Karganilla, 1972) about the edaphic algae of the Tropics. Moreover, quantitative information relating to soil algal abundance has been essentially non-existent.

The recent quantitative studies of the edaphic and litter algae and of the soil arthropods which were initiated in Hawaii Volcanoes National Park in conjunction with the International Biological Program (IBP) spurred interest in studying the possible role algae might have as food for soil dwelling insects. The quantitative results of both the algae and soil arthropods were derived from the same samples taken along a transect stretching between 4000' and 8000' on the island of Hawaii.

After cursory examination of some initial quantitative data, as well as having noted the co-occurrence of both Collembola and algae in the same environment, it was decided to investigate the possibility of a food relationship. Little is known of the natural food habits of such soil insects in nature, but algae have been suggested as a possible food source for the Collembola.

The suborder of Collembola to which Onychiurus belongs, the Arthropleona, has been recognized by several authors as being cosmopolitan in its geographical distribution. Most often these forms have been associated with edaphic habitats. Wallwork (1970) suggests

several factors which affect the distribution of these small, cutaneal-respiring insects and includes as examples moisture content of the soil, soil type and microfloral composition. He also summarized field studies and reported that the largest Collembola populations were found in the fermentation zone, where the greatest amount of decomposition of organic material was occurring.

Certain species of Collembola may play (Butcher et al., 1971; Wallwork, 1970) a role in nutrient cycling and decomposition as related to metabolism in the soil. In these insects the diet, as well as the rate of decomposition in the gut, seems to be dependent upon the quality and quantity of gut microbial flora. Palissa (fide Christiansen, 1964) stated that Collembola have a "strong tendency" to eat their own fecal material. It is suggested by one author (Christiansen, 1964) that food materials may be ingested by individuals several times with the eventual result being their decomposition.

Studies of the food habits of soil microarthropods in the laboratory are numerous but have been generally recognized as not producing results indicative of the diet in nature. Wallwork (1970) stated that the rate of reproduction in laboratory populations is influenced by the nutritional state of the organism. Data regarding hatching time of Collembola eggs have been collected from various sources by Thibaud (fide Butcher et al., 1971) and they indicate a range of 5 to 15 days for hatching at temperatures between 23 and 25°Centigrade. In one laboratory study involving feeding algae to soil microarthropods, the immature stages of four genera of oribatid mites were cultured (Sengbusch, 1954) and one, Galumna, "responded

successfully" to a diet of Protococcus. Diets of Collembola in culture experiments include (Wallwork, 1970) fungal hyphae and spores, bacteria, decaying plant material, unicellular algae, feces, living prey, etc., while it has been reported that Collembola inhabiting the soil feed on decayed or undecayed plant material, fungal mycelia and spores, bacteria (Christiansen, 1964) and algae (MacNamara, 1924). Many observed natural diets have been reported in general reviews of the literature (Christiansen, 1964; Butcher et al., 1971) concerning the Collembola, and there appear to be five possible categories of food preferences. These include fungal, bacterial, fecal, decayed or undecayed plant material and algal. Further information regarding any relationship between algae and species of Collembola is scarce.

Onychiurus folsomii, as a member of the order Collembola, was initially selected for study because it is a numerically predominant component (Radovsky, unpublished material) of the soil environment along the length of the IBP transect. The Collembola are commonly known as springtails, however, this particular species lacks a furcula. As described by Ashraf (1969), they are primarily soil-dwelling insects and the adults reach a maximum length of about 5 millimeters. The body is elongate, visibly segmented, cylindrical and white in color. There are six abdominal segments with the abdomen rounded posteriorly and without anal spines. Eyes are absent, the mouth parts are entotrophic, pseudocelli are present and a ventral tube is present on the first abdominal segment. These insects lack a tracheal system and have cutaneous respiration.

Due to the lack of information of the algae as a food for Onychiurus it was important to first determine if there was even the possibility that algae would be ingested by the insect if the algae were present as a food source. Once this was established it would be important to determine if there was evidence of algal ingestion in the field. And finally it was necessary to determine the ability of algae to support population growth and reproduction as evidenced by assimilation of algal products into the insect's tissues. Since it was well known that cell products are released (Hellebust, fide Stewart, 1974) by algal cells in varying amounts, depending on conditions such as physiological and environmental factors which affect the permeability of the membrane, it was thought important to incorporate this aspect into the analysis of algal utilization by the insect.

It is difficult to determine precisely the usual dietary qualities or quantities for an insect in nature and no definitive method, with the possible exception of gut analysis (Butcher et al., 1971) has been published. However, there seem to be several ways to approach the problems of feeding and food utilization. It was thought appropriate then to limit the present study to demonstrating the tenability of three hypotheses concerning the Collembola and the algae. These are: 1) that algae are ingested under laboratory conditions, 2) the likelihood that algae are ingested in natural habitats and 3) the likelihood that ingested algae are actually utilized.

MATERIALS AND METHODS

Algal Collection and Culturing

Qualitative as well as quantitative samples of the algae from soil, litter and dropped leaves were obtained each month for a period of 12 successive months at established sites on a transect in acquiring materials for the present study. The same materials or samples were utilized by the International Biological Program (IBP) on Mauna Loa (Fig. 1), a mountain on the island of Hawaii. Specifically, soil cores and fallen leaf samples were subsamples of those used by others to quantify the soil arthropods.

In the method developed to obtain quantitative algal data, and subsequently the algal stock cultures used in this study, a plexiglas rod with a .42 cm^2 sampling surface coated with polyethylene glycol ("Carbowax #1540") was used. The sampling surface was touched to soil, litter or leaves, and each rod was then replaced in the screw-capped vial in which it had been sterilized. Other algal samples were obtained from 9 cm soil cores in which a volume of soil was put in a sterile vial. The vials were placed in a styrofoam container in an attempt to minimize the temperature changes. A known quantity of sterilized, liquid Bold's Basal Medium (= BBM in the following text; see Table I for composition) was added to each vial using a burette within 24 hours after field collection. The vials were then sonicated, seven at a time[1], for 2 to 3 minutes. The vial was shaken slightly to uniformly suspend the material and using a sterile, plastic graduate pipette, 0.5 ml was removed and

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[1]Electromation Components Corporation, Model LP-1HD, 115 v, 50/60 cycles.

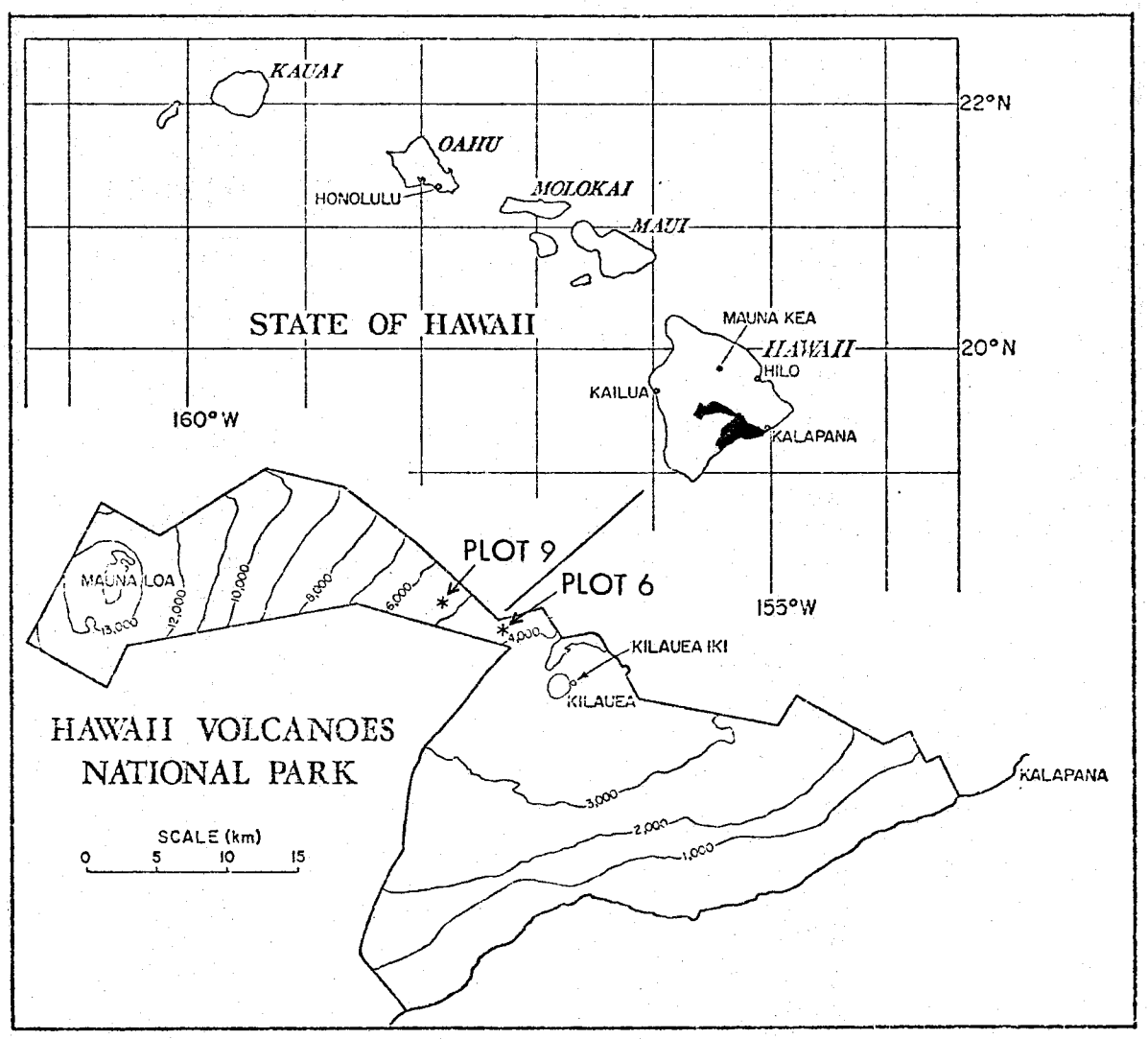

Fig. 1. Map of Hawaii Volcanoes National Park. Collection sites at 4200' and 5400', Plots 6 and 9, respectively, are indicated on Mauna Loa.

Table I. Composition of 3N Bold's Basal Medium
(Brown and Bold, 1964)

I.	Macro stocks (add 10 ml of each of the following per liter of final medium)	
	$NaNO_3$	30.0 g/400 ml glass distilled water
	KH_2PO_4	7.0 g/ "
	K_2HPO_4	3.0 g/ "
	$MgSO_4 \cdot 7H_2O$	3.0 g/ "
	$CaCl_2 \cdot 2H_2O$	1.0 g/ "
	NaCl	1.0 g/ "
II.	Micro stocks (add 1 ml of each of the following per liter of final medium)	
	EDTA	50.0 g EDTA acid and 31.0 g KOH/liter glass distilled water
	H-Fe	4.98 g $FeSO_4 \cdot 7H_2O$/liter acidified waters:
	H-Boron	11.42 g H_3BO_3/liter glass distilled water
	H-H	8.82 g $ZnSO_4 \cdot 7H_2O$/liter acidified water
		1.44 g $MnCl_2 \cdot 4H_2O$/ "
		0.71 g MoO_3/ "
		1.57 g $CuSO_4 \cdot 5H_2O$/ "
		0.49 g $Co(NO_3)_2 \cdot 6H_2O$/ "

*Acidified water = 1 ml concentrated H_2SO_4/liter glass distilled water.

put on a sterile BBM agar plate. The plates were placed under incubation lights for 3 weeks and then examined under the 12X power of a dissecting microscope.

Incubation lighting consisting of two 40-watt cool white fluorescent tubes was secured 40 cm above the table level. The lights were on continuously at an intensity varying between 300 and 400 foot candles over the length and width of the table. The mean temperature of the room was 27°C varying no more than 2 degrees.

Each algal species, or at least genus, has distinctive and recognizable colony characteristics. Using these criteria, the colonies of a single species were counted, recorded, identified and transferred using sterile techniques to a small stock culture plate which was incubated under lights and then put in a 15°C room for storage. Each algal colony was assumed to have been initiated (Appendix A) by a single cell. Each stock culture plate utilized in the following series of experiments was unialgal and only those species found at plots 6 and 9, 4200' and 5400' respectively were used.

Insect Collection

Onychiurus folsomii (Fig. 2) were collected from the soil by taking soil cores to a depth of 9 centimeters. The cores were placed in plastic bags and taken to the laboratory for extraction in a Berlese funnel. All soil arthropods which were negatively sensitive to heat or light and could pass through two wire meshes (ca. 1.5 and 6 mm respectively) were captured on a 100 X 15 mm

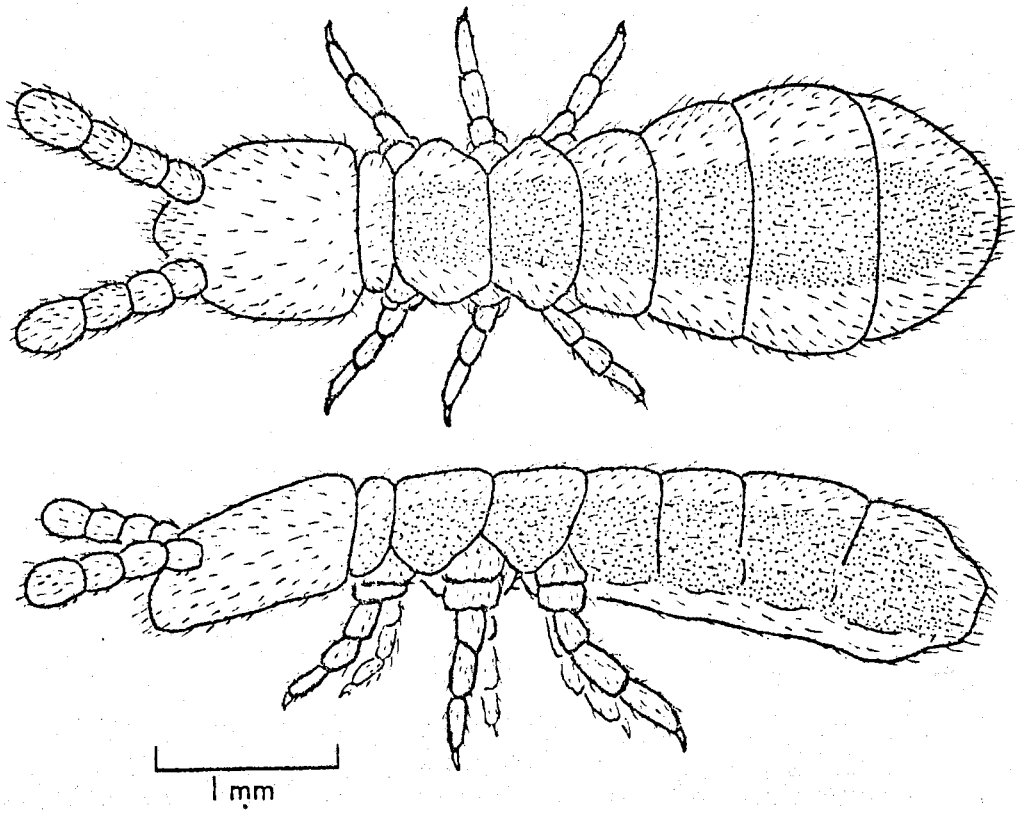

Fig. 2. Onychiurus cf folsomii. a. dorsal view; b. lateral view. Heavily stipped areas indicate the gut outline.

plastic petri dish containing BBM agar, which was placed directly under the funnel opening. It was decided that it was best to capture and retain the insects on the agar surface as its moist and relatively hard surface appeared to facilitate survival of O. folsomii in the laboratory. All animals other than O. folsomii were removed from the dish. These dishes, containing dropped soil particles, served as holding containers for the insects. Sterilized distilled water was added to them periodically to maintain a high relative humidity.

Algal Ingestion in the Laboratory

Unialgal Sprayed Plates

To demonstrate ingestion of algae in the laboratory it was necessary to have a supply of various unialgal cultures. A quantity of an algal species from a stock culture was transferred to a sterile shell vial containing 2.0 ml sterile BBM and 0.1 ml Tween 80 (polyoxyethylene [20] sorbitan monooleate). The vial was sonicated for 3 minutes. A capillary pipette was then placed in the vial and, using a jet stream of air, some of the algal suspension was sprayed onto a sterile, 50 X 12 mm, lock-top, BBM agar petri plate. Three replicate plates were sprayed for each species stock culture used and were incubated under the lights to allow the algae to grow for at least 2 weeks.

Algal Ingestion

To record ingestion, microphotographs of algal-fed Onychiurus folsomii were made. To do this, the insect was put on a dry

microscope slide and a plastic cover slip was slowly lowered onto it without additional pressure which would have squashed the insect. Thus a preparation was obtained in which there was an opportunity to see any algal cell structure within the animal, and changes in that structure taking place from the mouth to the anal openings.

Algal Growth from Feces

In order to determine if the ingested algal cells were destroyed or still viable after passing through the gut, two things were done. First, several individuals of Onychiurus folsomii were placed on separate agar plates each containing a single algal species. After the guts were full from feeding on the algae, the insects were removed to a sterile agar plate to excrete. This plate containing the fecal material was placed under incubation lights. Secondly, squashes of insects fed different species of algae were made on an agar plate. All the plates were periodically checked for growth of algal colonies.

Algal Ingestion in Nature

In order to determine if Onychiurus folsomii ingests algal cells in its natural soil environment several field collections during an 8 month period were made. Each core was placed in a Berlese funnel within approximately 45 minutes of collection. Four procedures were used to test for algal presence in the gut. The first was direct observation of the somewhat transparent gut using 10X power. This was done within 15 minutes of the insects' falling

onto the agar plate. It was possible to observe the gut contents of the animal and to distinguish colors through the somewhat transparent cuticle, e.g. as the greenness of algae. The second procedure involved squashing a number of freshly-collected insects with a sterile inoculating needle onto a BBM agar surface in a small petri dish which was then placed under incubation lights within 18 hours of the "inoculation". Another procedure involved placing a number of freshly-collected insects on a sterile BBM agar plate. After defecation of gut contents the insects were removed using a camel's hair brush and as before the plates were put under incubation lights. Direct microscopic examination of gut contents from squashed preserved and unpreserved insects was also tried.

Algal Utilization

Onychiurus Growth and Reproduction on Algal Plates

To determine if ingested algae were actually utilized, three experiments were designed. The first involved feeding the Onychiurus folsomii different species of algae. Plates of algal species sprayed as described previously were used for these experiments. Various numbers of visibly empty-gutted insects were added to the different unilagal plates. Since the numbers of O. folsomii collected were limited, a small number of insects was initially added to each plate. At various times during the experiments an additional supply of O. folsomii would be collected and so then, in most cases, 10 more individuals were added to some of the feeding plates. Replicate plates were sometimes made.

Differing from the algal incubation lighting, the light intensity reaching the table surface in the room containing the petri dishes for feeding experiments was 50 foot candles. That reaching the algal surface and insects was reduced by water droplets formed on the inner surface of the petri dish lid. During each week the lights were on 10 hours per day 5 days, 4 hours on the sixth day, and dark on the seventh. The mean temperature of the room was 24°C varying no more than 2 degrees.

Using a dissecting microscope the insects were counted or observed periodically on the different plates for feeding behavior, numbers of individuals moulting, egg deposition, larvae and death.

Population Growth and Reproduction on BBM Agar Plates

To determine the possibility of the utilization of the medium as a food source, tests were run to determine the survival of the insects on BBM agar as the only food source. It was also decided to use agar, without the addition of salts, and inorganic silica gel. All substances would serve as both food sources and growth surfaces. Ten empty-gutted Onychiurus folsomii not previously fed on algae, were put on each of three replicate plates of sterile BBM agar, sterile agar and silica gel. The silica gel was prepared from sodium silicate and rinsed to remove sodium chloride and excess hydrochloric acid. Each of the nine plates was periodically examined for moults, eggs and live individuals. Sterilized distilled water was added every 3 days or so to maintain a high humidity within all the plates.

Carbon-14 Tracer Techniques

In an attempt to determine if the algae ingested by the insects were actually assimilated rather than passed through the gut unchanged, a radioactive tracer experiment was designed using carbon-14. Small petri dishes sprayed with unialgal cultures and grown under incubation lights for 2 to 3 weeks were employed by marking the algae with carbon-14. Empty-gutted insects were exposed to the labelled algae and after varying periods of time the resultant empty-gutted insects and their fecal material were both analyzed for radioactivity. The labelled carbon dioxide was generated by adding 1 ml labelled barium carbonate to .1 ml 1N hydrochloric acid in a small rubber-capped serum vial.

Two methods were devised to introduce the labelled carbon dioxide to the algae but the first (Appendix B) proved unsatisfactory. The successful method involved putting three to five uncovered unialgal petri dishes in a lypholyzing flask. After a partial vacuum was created, a vial containing labelled carbon dioxide was attached to a flask opening and the gas was sucked into the flask. The algal cells were in the presence of the labelled carbon dioxide for varying periods of time between 36 and 48 hours. The plates were removed from the flask and put under a hood exhaust with lids intact. Material to be assayed for radioactivity was put into a liquid scintillation counting vial containing .1 ml 30% hydrogen peroxide and .1 ml perchloric acid. The vial was heated to 60°C then placed in an ice bath. Five ml "Aquasol" counting solution was added to

the vial in which the radioactivity was then measured in a Packard, "Tri-Carb" liquid scintillation spectrometer, Model 2420.

Counting efficiency (no. of observed counts/no. of disintegrations in the sample) is dependent according to Bush (1964) on the physical and chemical nature of the sample and decreases through quenching. Efficiencies for the counts per minute obtained were derived from the channel ratio standard for this particular machine. The ratio, as printed out with the sample number and corresponding counts per minute (cpm), has a comparable efficiency standard for carbon-14 (Fig. 3). Actual efficiency is extrapolatable from the A.E.S. ratios, and was generally 55 to 65% throughout the experiments. The cpm may be corrected then to 100% efficiency. To further facilitate the comparison of number, counts were corrected (except where noted) to the initial number of insects at the beginning of the experiment. Machine variances of each sample count are given.

Three experiments were carried out using this method of labelling the algae in the lypholyzing flask. In the first experiment on October 10, 1973, two species of algae, Tetracystis aplanosporum, 30775, and Chlorococcum isabeliense, 30804, both collected August 21, 1973, and a sterile BBM agar plate were exposed to labelled carbon dioxide for 46 hours. Twenty insects collected August 21, 1973, from Plot 6 were put on each of the three labelled plates. Twenty-two and one-half hours later 20 insects from 30775, 18 insects from 30804 and 18 insects from the labelled sterile agar were removed to sterile BBM agar plates for elimination of the gut to occur. When the insect gut was no longer green as seen under the

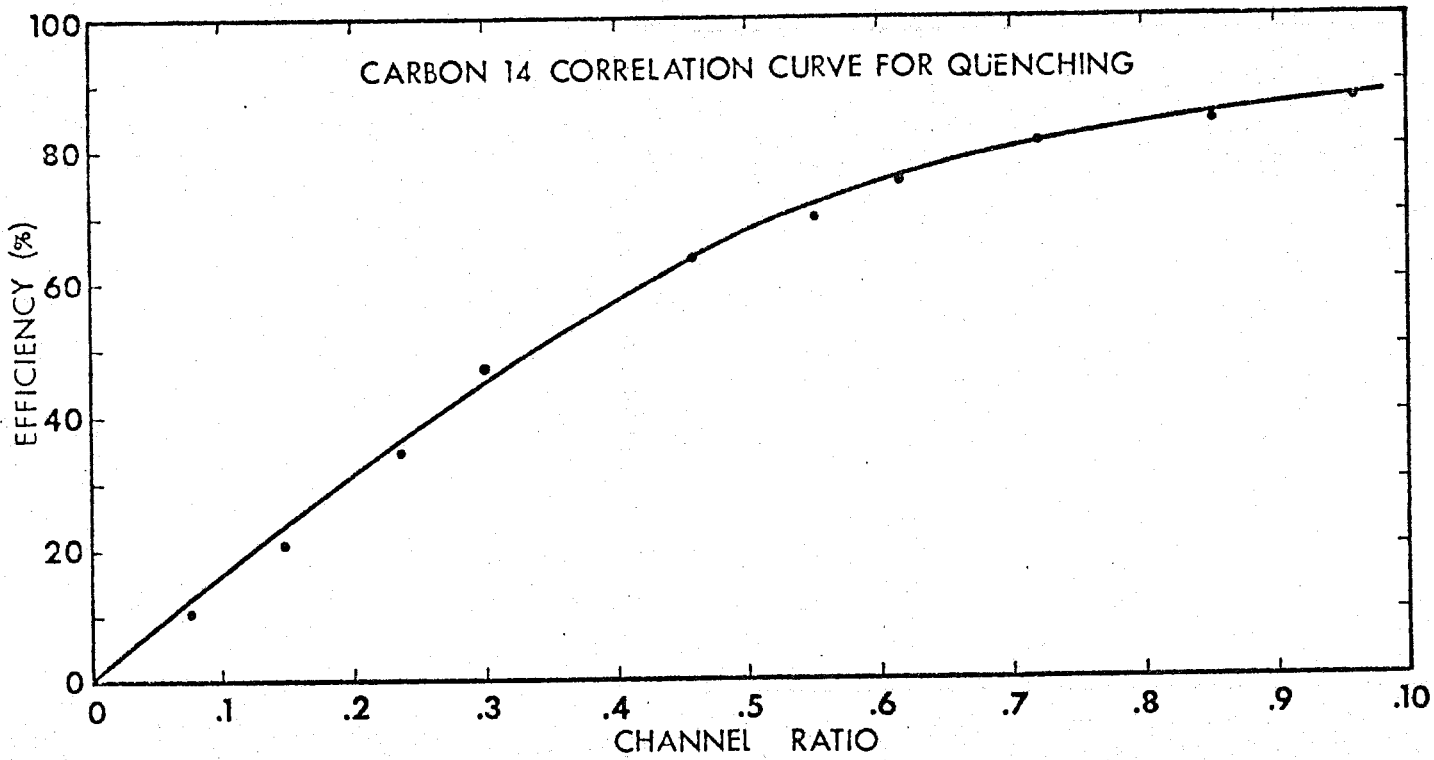

Fig. 3. Channels ratio versus efficiency curve for Carbon-14 quenching.

12X power of a dissecting microscope, it was placed in a counting vial. The fecal material was scraped or cut from the agar and put in a separate counting vial. The labelled algae were scraped in a semi-quantitative way to determine the total labelling count, the main purpose being to see if it had in fact been labelled, and were added to a similar counting vial. From the "elimination" plate five of the empty-gutted insects from 30775 and five from 30804 were put on "cold", i.e., non-labelled 30775 and 30804 replicate plates, respectively, in order to determine length of time labelled material stayed in the insect. In 23 hours both sets of five insects had full guts and were removed to two sterile BBM agar petri plates for defecation. The material was handled as described above to determine radioactivity.

The second experiment was to determine if the amount of radioactivity in the insect was affected by feeding time or exposure to a labelled algal plate. Twenty insects were put on each of four algal plates which had been exposed to a carbon-14 atmosphere for 36½ hours. The plates included Diogenes bacillaris, 30682, collected July 24, 1973; Lyngbya sp., 30718, collected July 24, 1973; Chlorococcum isabeliense, 30912, collected September 16, 1973; and Chlorhormidium flaccidum, 30927, collected September 16, 1973. The following procedure was applied to each of the four plates. After 1 hour, five full-gutted insects were removed and put on a sterile plate to defecate, after which the empty-gutted insect and fecal material were put in separate vials and treated as before to determine the radioactivity. In another 3 hours, or after a total of 4 hours

exposure and feeding on the labelled algal plate, five more insects were removed to a sterile plate to defecate. The entire procedure was repeated for each algal species after a total of 8 hours feeding and a total of 21 hours feeding.

The final experiment was to determine if assimilation of labelled material into the Onychiurus individual is increased with time after an initial exposure and then removal from the label. The algal species used were replicates of those plates used in the previous experiment. Ten or more insects were put on each labelled plate which had been exposed to the "hot" carbon dioxide atmosphere for 48½ hours. Within 45 minutes the Onychiurus were put on the labelled plates and left for 1 hour. At that time all were removed to sterile BBM agar plates. After 1 hour, three with empty guts were removed and the insects and their fecal materials were placed in separate counting vials. This procedure was repeated using the other individuals after 4 hours and 8 hours.

Algal Contamination of the Onychiurus Body

To assess if algal contamination on the insect body surface would affect the experimental results, algae were gently scraped across the back of three insects with a broom bristle. They were examined using 50X power for presence of green on their carapaces and legs. The insects were then placed in a vial containing BBM and the vial was sonicated for 1 minute. Since green was still present on the cuticle after 2 minutes, the insects were returned to the sonicator for another 2 minute exposure. They were removed and

observed under 50X power and this time were found to have clear and algal-free carapaces and legs. The BBM from the vial, presumably containing algal cells was poured onto a sterile BBM agar plate. A control plate containing BBM was inoculated and both plates were incubated. Observations of algal growth were made periodically. The experiment was repeated using 10 insects, leaving the vial in the sonicator for 3 minutes without interruption. Also, observations of the carapace of insects walking on algal colonies using 12X power were made; and observations of the carapace of squashed insects were made using 43X power.

Extrapolation to Nature

The next most obvious problem, for which only a partial answer can be obtained within the scope of this study, considered whether there was a sufficient algal population present in the field to support the Onychiurus population. It was necessary to determine not only the feeding and the gut-evacuation rates, but sizes of algal cells and the size of the insect gut to get an estimate of the number of cells that could be contained in an "average" insect.

In a partial attempt to obtain such an estimate, observations were made of the time needed for an empty-gutted insect to obtain a full gut, as well as time for the evacuation of that material. Using a freezing microtome, cross sections of full-gutted insects embedded in agar were made to obtain an idea of the shape of the gut. To obtain algal cell volumes the following procedure was used. From the remaining algal suspension used to spray the feeding plates,

1 ml was added to a vial containing ½ ml of 10% formalin. Observations of cell and filament shape and dimensions were made on 25 cells from each series of sprayed culture plates. The average volumes of these cells were calculated. For further calculations it was assumed the gut of a .4 cm animal was cylindrical, was 3/4 the total length of the insect and occupied a diameter of ½ mm in the body cavity.

RESULTS

It was apparent from the algal and Collembolan field collection data (Figs. 4 and 5; Appendix A) that the organisms co-occurred. Under laboratory conditions, colonies of the various algal species growing on the sprayed plates were walked over, probed and ingested by the insect. Table II is a listing of algal species found at two sites on Mauna Loa and ingested by Onychiurus folsomii in the laboratory.

Algal Ingestion in the Laboratory

Microphotographs (Figs. 6 and 7) show the resultant visible green "rod" in the insect gut after placing Onychiurus individuals on an algal plate. Changes in algal cell structure throughout the length of the gut could not be adequately discerned.

Algal Growth from Feces

Table III shows the results of the growth of fecal material and insect squashes from full-gutted algae-fed Onychiurus folsomii. No algal colonies from fecal material of insects fed on Anabaena sp. or Chlorococcum isabeliense grew, but fungal and bacterial colonies were noted. Squashes were not made of insects fed Anabaena sp. or Diogenes bacillaris. Algal growth from squashes of the insect occurred however in all four species tested.

Algal Ingestion in Nature

At no time was a green gut, which could be taken to indicate algal presence, observed in Onychiurus folsomii collected from the

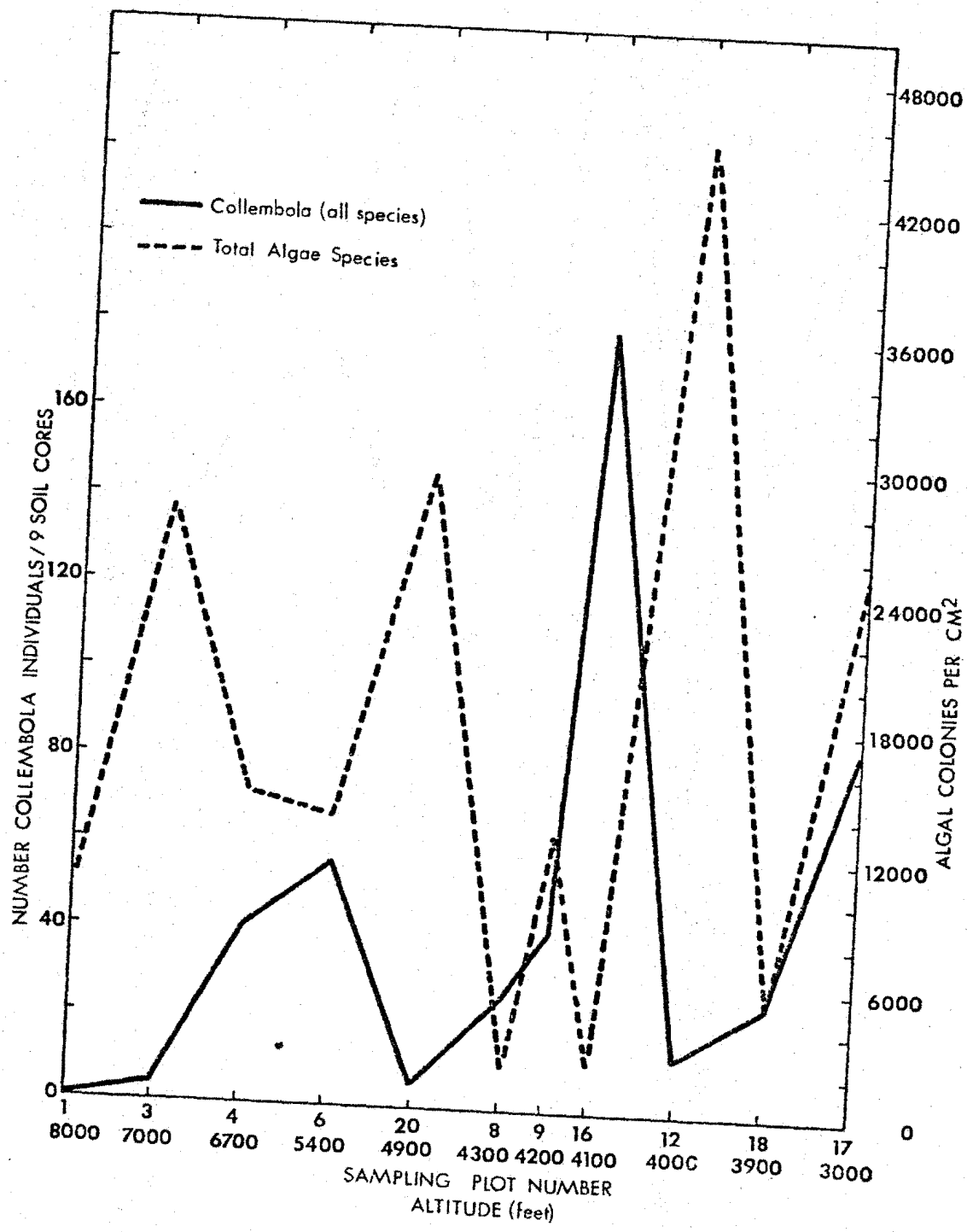

Fig. 4. Total numbers of all Collembola and algal species (colonies/cm^2) collected from 9 cm soil cores from various IBP plot sites in December 1972.

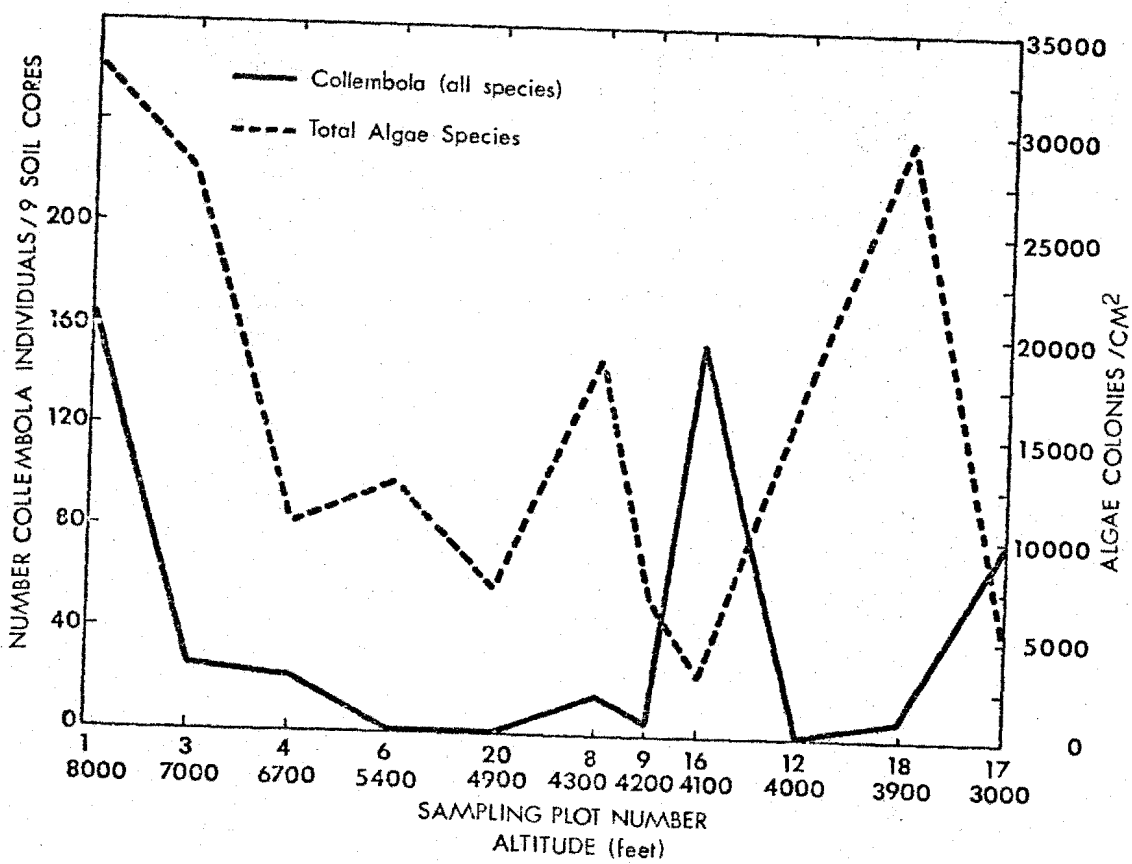

Fig. 5. Total numbers of all Collembola and algal species (colonies/cm^2) collected from 9 cm soil cores from various IBP plot sites in November 1972.

Table II. Algal species ingested by Onychiurus folsomii with culture numbers and collection dates. The asterisks indicate those algal stock cultures used for laboratory growth experiments.

Algal species	Stock culture no.	Collection date
Chlamydomonas sp.	30791	21-VIII-73
*Chlorella sp.	30790	21-VIII-73
*Chlorhormidium flaccidum (Braun) Fott	29976	XII-72
*Chlorhormidium flaccidum	30647	24-VII-73
Chlorhormidium flaccidum	30740	24-VII-73
Chlorhormidium flaccidum	30927	16-IX-73
*Chlorococcum isabeliense Archibald and Bold	29967	XII-72
*Chlorococcum isabeliense	30667	24-VII-73
Chlorococcum isabeliense	30804	21-VIII-73
Chlorococcum isabeliense	30912	16-IX-73
*Chlorococcum minutum Starr	30414	22-V-73
*Chlorococcum minutum	30662	24-VII-73
*Chlorococcum paludosum Archibald and Bold	30791	21-VIII-73
*Chlorococcum reticulatum Archibald and Bold	30650	24-VII-73
*Diogenes bacillaris (Naumann) Pennington	29168	XII-72
Diogenes bacillaris	29331	XII-72
*Diogenes bacillaris	30450	22-V-73
*Diogenes bacillaris	30665	24-VII-73

Table II. (Continued) Algal species ingested by *Onychiurus folsomii* with culture numbers and collection dates

Algal species	Stock culture no.	Collection date
*Diogenes bacillaris	30682	24-VII-73
*Stichococcus sp.	30410	22-V-73
*Tetracystis aplanosporum (Arce and Bold) Brown and Bold	30501	22-V-73
*Tetracystis aplanosporum	30775	21-VIII-73
Tetracystis aplanosporum	30838	21-VIII-73
*Anabaena sp.	30433	22-V-73
Lyngbya sp.	30718	24-VII-73
*Nostoc sp.	29303	XII-72
*Oscillatoria sp.	30498	22-V-73
Oscillatoria sp.	30653	24-VII-73
Oscillatoria sp.	30735	24-VII-73
Trentepohlia sp.	30499	22-V-73
Trentepohlia sp.	30657	24-VII-73

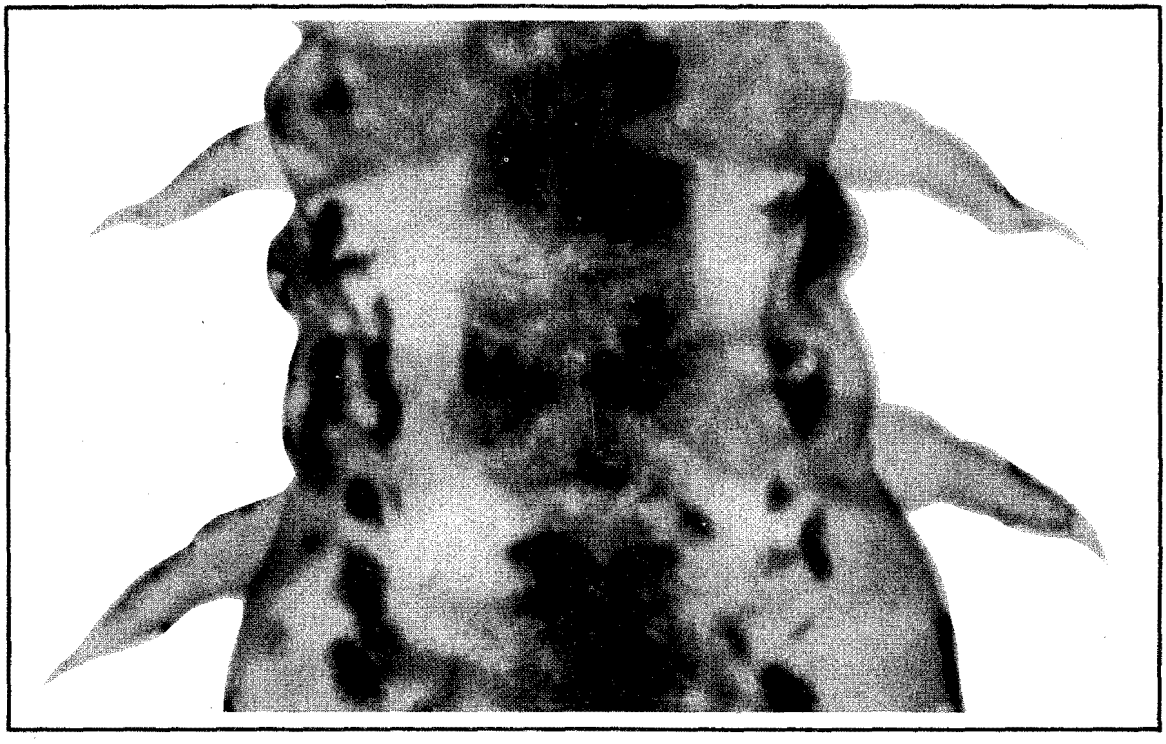

Figure 6. Ingested Chlorococcum isabeliense in the midgut of Onychiurus folsomii. One centimeter equals 0.8 millimeters.

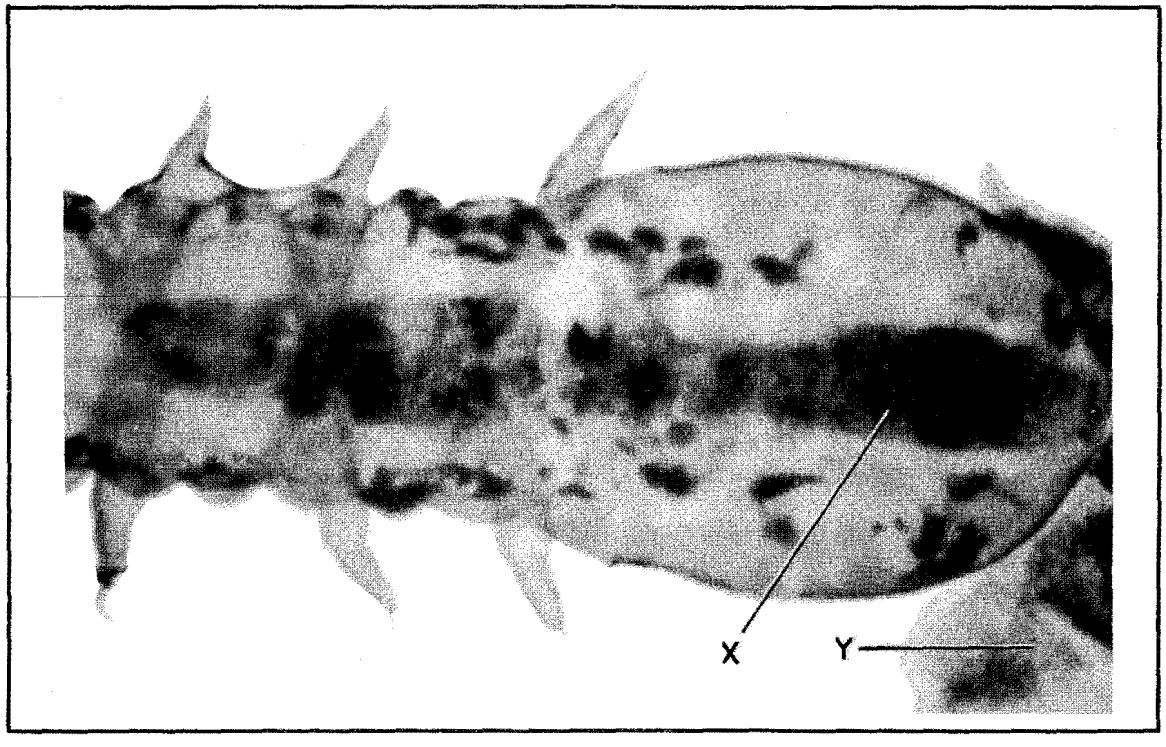

Figure 7. Ingested Chlorococcum isabeliense showing nearly throughout the length of the Onychiurus folsomii gut. One centimeter equals 2 millimeters. The concentration of algal cells shows at "x" in the hindgut and as excreted material, "y", at the right.

Table III. Fecal and gut analyses of Onychiurus folsomii when fed different species of algae. The results are in terms of the number of insects fed and the total number of algal colonies resulting on the plates. Culture numbers are directly below each species name.

Algal species	Fecal analysis		Gut analysis	
	Insects	Colonies	Insects	Colonies
Anabaena sp. 30433	1	0	--	--
Chlorhormidium flaccidum 29976	2	6	3	5
Chlorococcum isabeliense 29967	4	0	3	2
Diogenes bacillaris 30450	15	7	--	--
Nostoc sp. 29303	3	19	3	13
Trentepohlia sp. 30499	3	32	3	3

field. Commonly, brownish gut contents or a clear gut were observed in the insects. Though 50 such insects from nature were squashed on BBM agar plates, no algal growth was observed even after 1 month's incubation. Likewise, no algal growth was observed on any of the five fecal plates containing fecal material from the 50 insects. No algal cells were observed with a microscope in squashed insects, fresh or preserved. No algal colony was ever observed on any of those agar plates used to capture and maintain the insects, which also contained the insect excreta and fallen soil fragments from the Berlese funnel. These plates containing the insects were kept in reduced lighting such as was described for the population growth experiments.

Algal Utilization

Population Growth and Reproduction on Algal Plates

Table IV is a listing of algal species with which sprayed plates were made and feeding and growth experiments conducted. It can be seen from this table as well as Table V that there was an overall increase in numbers of insects for all these species with the exception of Anabaena sp., Chlorella sp., Chlorococcum paludosum and Oscillatoria sp. which showed negative or no increases in population. Two types of indices were calculated, one based on the increase of individuals and another on the increase of moults. The first index gives an assessment of the increase in the population on various algal species. To obtain this terminal index (Table V), the total number of insects added to each plate was

Table IV. Results of feeding, growth and reproduction experiments of Onychiurus folsomii with algal species. Ten additional insects were put on some plates after the experiment had started and this is indicated in the fourth column. Numbers of insects, moults and presence of eggs (x) on the plates are also indicated. Stock culture numbers, with replicates, are in parentheses. Total number of moults in parentheses were those counted on the final day of the experiment while the others were counted 18 days prior to the termination of the experiment.

Algal species	Insect numbers				Total no. days	Total no. mounts	Eggs present
	Initial	Max seen	Final	Day added			
CHLOROPHYTA							
Chlorella sp. (30790)	10	10	6	--	53	20	-
Chlorhormidium flaccidum (29976)	5	39	39	51	104	95	x
Chlorhormidium flaccidum (30647-1)	5	35	35	9	62	43	x
Chlorhormidium flaccidum (30647-2)	5	30	26	9	62	52	x
Chlorococcum isabeliense (29967-1)	1	10	7	9	62	22	-
Chlorococcum isabeliense (29967-2)	4	29	19	--	104	34	-
Chlorococcum isabeliense (30667-1)	5	15	9	9	62	52	-
Chlorococcum isabeliense (30667-2)	5	26	26	9	62	54	x
Chlorococcum minutum (30414)	4	65	65	51	104	52	x
Chlorococcum minutum (30662-1)	10	53	17	--	53	33	x
Chlorococcum minutum (30662-2)	5	28	28	9	62	55	x
Chlorococcum paludosum (30791)	10	10	2	--	53	28	-
Chlorococcum reticulatum (30650)	10	27	25	--	53	47	x
Diogenes bacillaris (29168-1)	4	73	73	--	101	87	x
Diogenes bacillaris (29168-2)	2	132	132	2	55	45	x
Diogenes bacillaris (30450-1)	4	11	2	49	102	59	-
Diogenes bacillaris (30450-2)	4	24	23	51	104	104	x
Diogenes bacillaris (30665-1)	5	15	9	9	62	56	x
Diogenes bacillaris (30665-2)	5	60	60	9	62	60	x
Diogenes bacillaris (30682)	10	20	13	--	53	30	x
Stichococcus sp. (30410)	4	47	47	48	101	83	x
Tetracystis aplanosporum (30501)	4	24	22	57	104	61	x
Tetracystis aplanosporum (30775)	10	15	15	--	53	30	x
CYANOPHYTA							
Anabaena sp. (30433)	4	4	0	--	49	(15)	-
Nostoc sp. (29303-1)	4	34	24	51	104	82	x
Nostoc sp. (29303-2)	10	63	63	--	55	58	x
Oscillatoria sp. (30498)	4	10	0	41	83	(22)	-

Table V. Population growth and moult indices of Onychiurus folsomii fed on different algal species. The indices represent averaged values for the number of experiments (N).

Algal species	N	Average index value for N runs		
		Terminal	Maximum	Moult
Oscillatoria sp.	1	-.169	-.048	.024
Chlorococcum paludosum	1	-.151	---	.080
Anabaena sp.	1	-.082	---	.121
Chlorella sp.	1	-.075	---	.057
Chlorococcum isabeliense	4	.040	.100	.087
Tetracystis aplanosporum	2	.086	.095	.069
Chlorhormidium flaccidum	3	.244	.265	.073
Chlorococcum minutum	3	.277	.504	.073
Chlorococcum reticulatum	1	.283	.321	.134
Stichococcus sp.	1	.327	.327	.071
Diogenes bacillaris	7	.503	.550	.106
Nostoc sp.	2	.530	.578	.113

subtracted from the final number of insects and divided by the total number of days of the experiment. These values are averaged for replicates of each species. The maximum index was obtained in the same way but the maximum number of insects observed (Table IV) was used in the calculation in place of the final number of insects.

If the moulting indices (moults/insects/days) in Table V are examined it can be seen that Chlorococcum reticulatum, Anabaena sp. Nostoc sp. and Diogenes bacillaris showed the greatest increases in numbers, respectively, as indicated by the number of moults. These figures were substantiated by the terminal indices and indicated an overall increase in numbers of individuals. Anabaena sp. was an exception however, perhaps due to the small number of experimental insects (4) on the plate. On 8 of the total 27 algal plates the number of moults decreased at some point in the experiment. On two occasions an insect was observed eating a moult. Since it proved difficult to obtain a consistent supply of Onychiurus individuals, when an additional supply was available, 10 insects were added to some of the feeding plates. These additional insects plus the initial number added was used for the calculation of the index.

Examples of the moulting (Fig. 8) of Onychiurus folsomii feeding on Diogenes bacillaris are characteristic of the success obtained in rearing this insect. Figures 9 and 10 are records of the survival and development of the insects using two replicates each of Diogenes bacillaris and Chlorococcum isabeliense. The similarity of moulting patterns on the C. isabeliense plates should be noted, despite the increase of individuals on one and decline on the other.

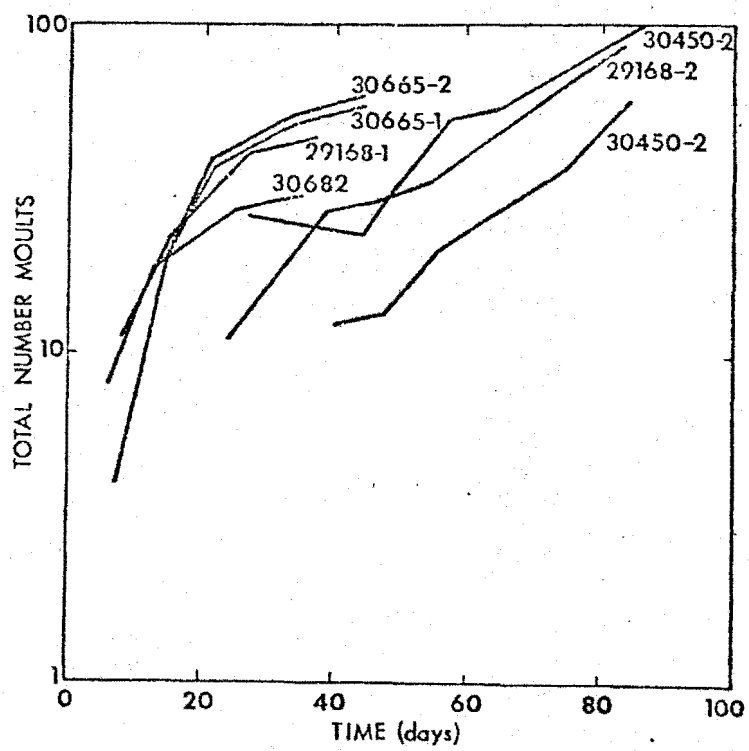

Fig. 8. Moults of all Onychiurus folsomii on different plates and strains of Diogenes bacillaris. Algal stock culture numbers and replicates are indicated.

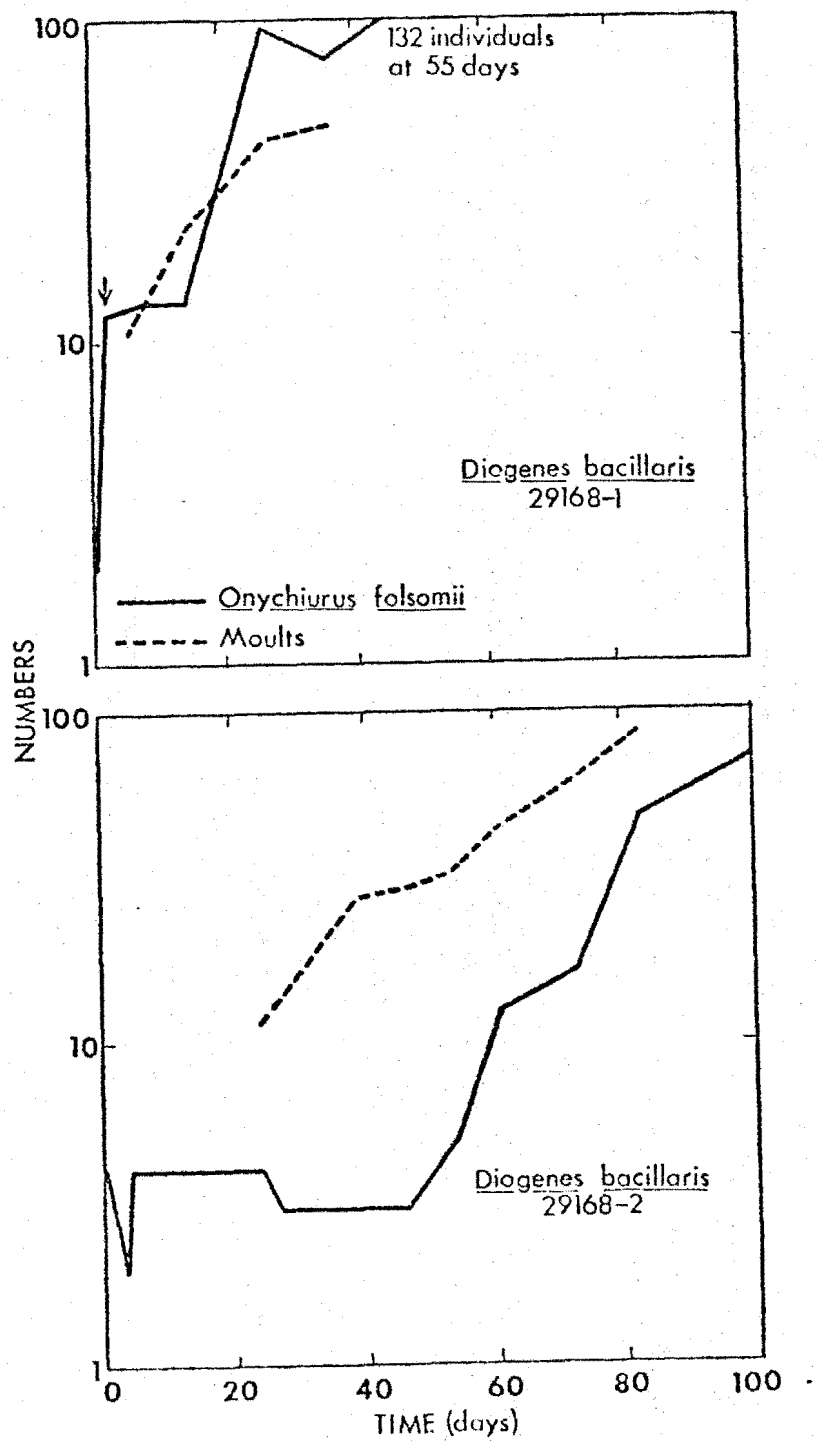

Fig. 9. Numbers of Onychiurus folsomii individuals and moults when fed on replicate Diogenes bacillaris plates. The arrow represents the addition of 10 insects to the plate.

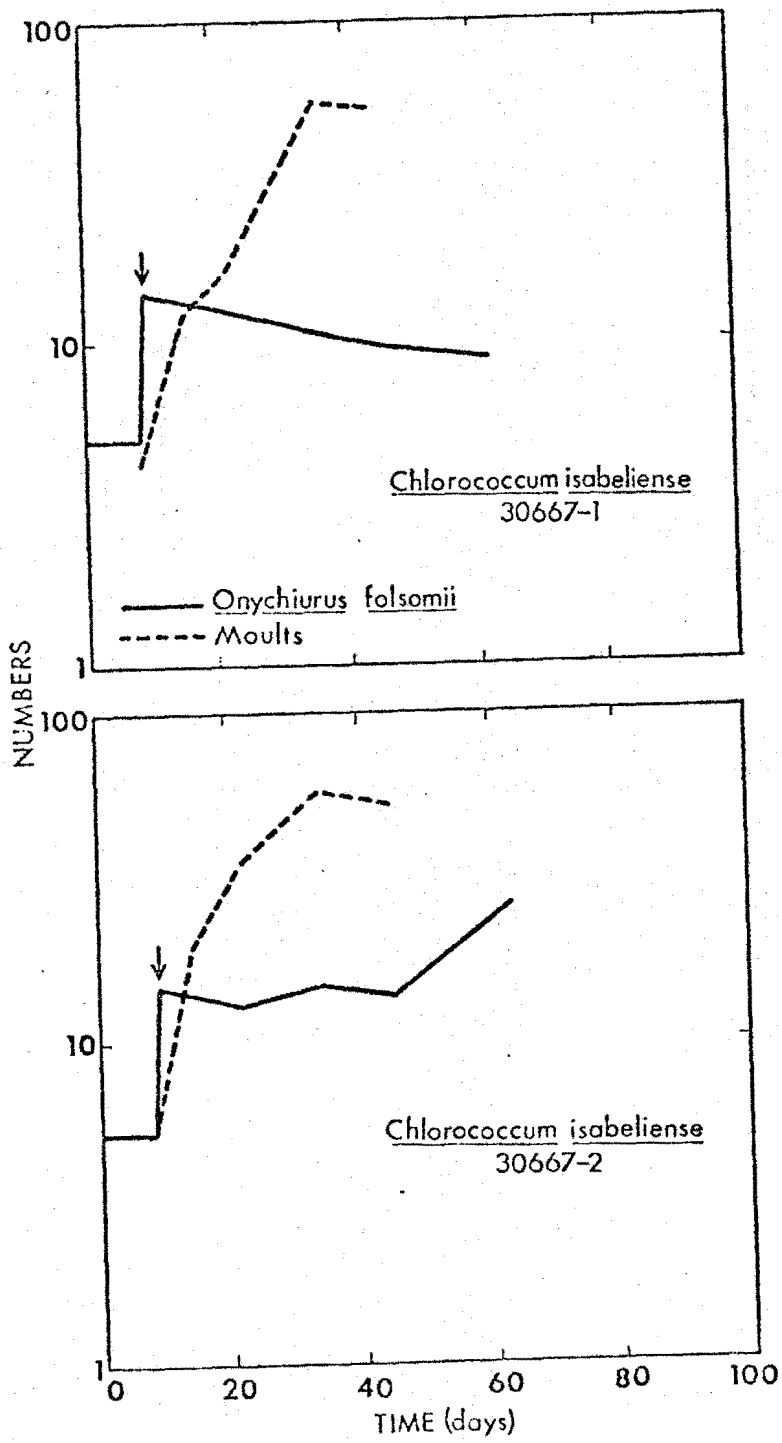

Fig. 10. Numbers of Onychiurus folsomii individuals and moults when fed on replicate Chlorococcum isabeliense plates. The arrow represents the addition of 10 insects to the plate.

Reproduction was evidenced not only by the presence of egg clusters on the plates, but also by the hatching of larvae. Table VI includes a few examples of algal plates on which eggs and larval insects were observed. The range of increase in individuals, which represents larval hatching, observed after eggs were noted was between 2 and 77. It was noted that with more than 10 insects on the plates, the mean hatching time of eggs on all algal species was 14 days. The mean hatching time regardless of numbers of insects on the plates was close to 16 days, while on plates containing 4 to 5 insects without the addition of 10 insects, the hatching time was 19 days.

Population Growth and Reproduction on BBM Agar Plates

An increase in numbers of individuals was observed on one agar plate (Table VII), and eggs were noted on two of the plates. Some population growth or increase in individuals also occurred on BBM agar (Fig. 11) but eggs were not seen. Mites (species unknown) were noted on two of the three replicate plates of BBM agar as well as the plain agar. One mite was observed eating an apparently live *O. folsomii*. Fungi were noted on one of the BBM replicate plates. There was no population growth of *O. folsomii* on the silica gel plates. Data for the replicate plates were retained in Table VII to illustrate the increase in insect numbers on one agar plate. Only one of the three replicate plates showed an increase in individuals however and this occurred after a period of 70 days.

Table VI. Observations of eggs and larvae of Onychiurus folsomii on plates containing different algal species. The numbers in parentheses denote the number of days since 10 additional insects were added to the plate.

Algal species	Eggs noted		After eggs noted	
	Initial number insects	Day of experiment	Hatching time (days)	Number insects increased
Chlorococcum isabeliense 30667-2	4	27	15	26
Chlorococcum minutum 30662-1	10	13	12	43
Chlorhormidium flaccidum 30647-1	5	15 (6)	19	2
Chlorhormidium flaccidum 29976	5	30	12	6
Chlorhormidium flaccidum 30647-2	5	34 (23)	10	15
Diogenes bacillaris 29168-1	2	8 (6)	19	77
Diogenes bacillaris 30450-2	5	27	30	5
Nostoc sp. 29303-1	5	64 (13)	12	9
Stichococcus sp. 30410	5	61 (14)	12	7
			$\bar{x}$=16	$\bar{x}$=21

Table VII. Observations of growth and reproduction of Onychiurus folsomii on petri dishes containing only agar, BBM agar or silica gel

Replicate	BBM					Agar					Silicate				
	Insects		Moults	Eggs	Days of experiment	Insects		Moults	Eggs	Days of experiment	Insects		Moults	Eggs	Days of experiment
	Initial no.	Final no.				Initial no.	Final no.				Initial no.	Final no.			
a	10	0	45	-	42	10	3	98	+	85	10	0	2	-	29
b	10	0	56	-	85	10	1	40	-	85	10	0	-	-	29
c	10	0	63	-	85	10	12	82	+	85	10	0	3	-	29
$\bar{x}$	10	0	55	-	71	10	5	73	+	85	10	0	3	-	29

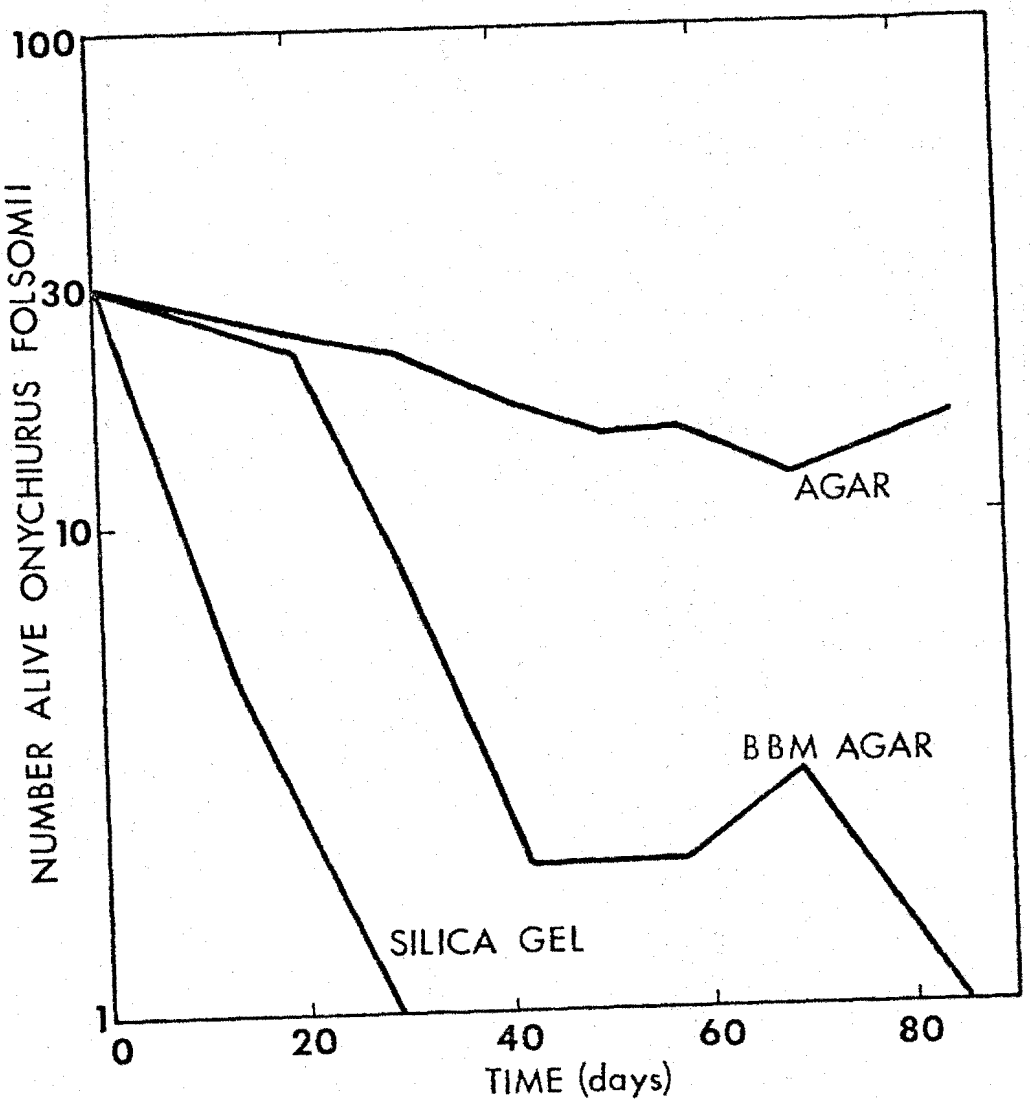

Fig. 11. Numbers of Onychiurus folsomii grown on plates of agar, BBM agar and silica gel. Each line represents the sum of three replicate plates.

Carbon-14 Tracer Experiments

In the first experiment using radioactive carbon dioxide the insects clearly showed ingestion and presence of labelled material (Table VIII) of Tetracystis aplanosporum, 30775 and Chlorococcum isabeliense, 30804. All counts are corrected for efficiency and for the original number of insects, if some were "lost". The fecal material could not be obtained from the subset of insects later exposed to unlabelled algal plates, but 80 to 87% of the labelled material was still present in the animals after almost 24 hours of not being exposed to any labelled algal material. The agar on the sterile agar plate had a total count of about 213 cpm. After a time the agar was noted to be pitted, indicating agar ingesting by the insects, and some radioactivity was found in these insects. Fecal material could not be obtained from the insects fed only on the agar.

Figure 12 and Table IX illustrate the presence of labelled algal material into the insect tissues and the accumulation with time. The label in the fecal material remained more or less constant with all four algal species and was significantly less than that present in the insects. Table X illustrates the variability of the results of the final experiment using carbon-14 as a tracer, but presence of labelled material in the insect after exposure to the algae is still indicated despite the high counting variance.

Algal Contamination of Onychiurus

Insects used to assess if algal contamination on the insect carapaces and legs would affect the experimental results were algal-free under 50X power after 3 minutes sonication. The agar plates

Table VIII. Fecal and insect carbon-14 counts and variances in parentheses resulting from labelled, algae-fed Onychiurus folsomii

Algal species	No. insects to plate (vial)	Corrected counts	
		Insect cpm; insect/fecal ratio below	Insect cpm*
Tetracystis aplanosporum 30775	20 (15)	18640 (.4) 27	16279 (.7)
Chlorococcum isabeliense 30804	18 (13)	22859 (.4) 102	18480 (.7)

*Five empty-gutted insects were transferred from labelled to unlabelled algal plates. Numbers are corrected to a standard 15 insects.

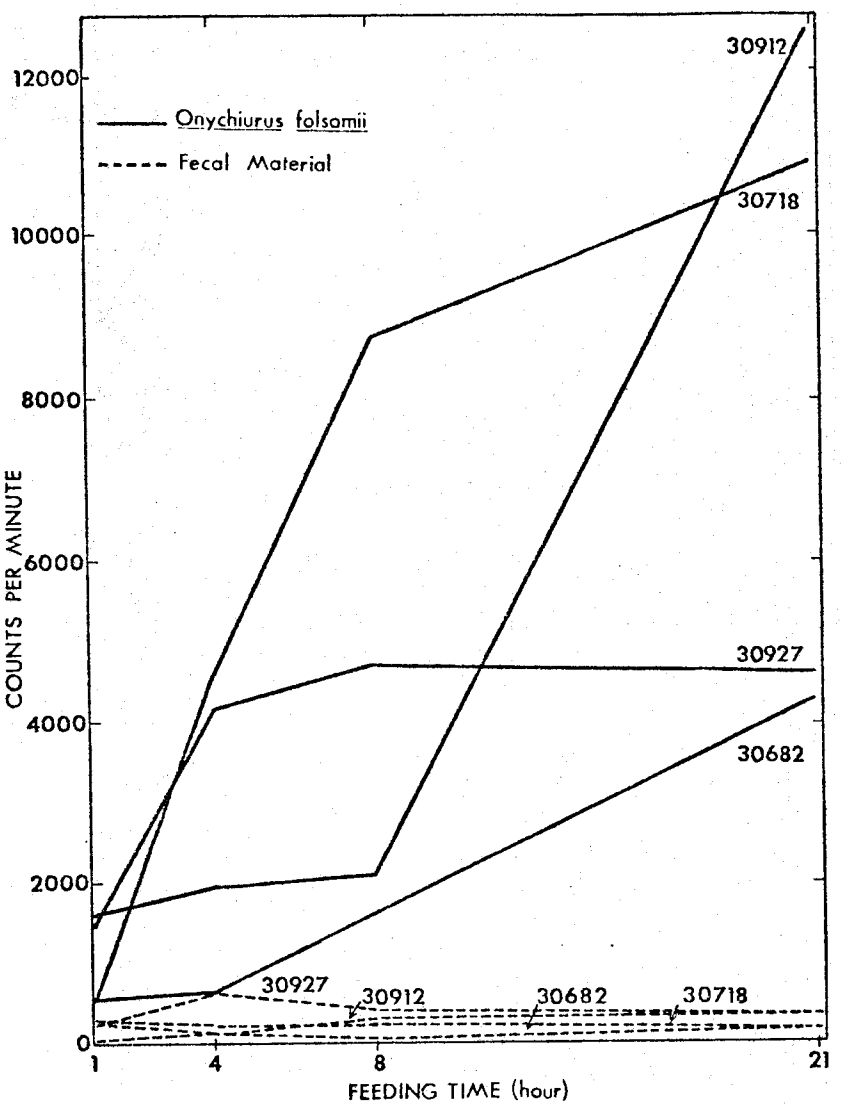

Fig. 12. Radioactive counts of insects and fecal material when fed on different labelled algal species for one hour, four hours, eight hours and twenty-one hours.

Table IX. Algal carbon-14-assimilation feeding experiments. Numbers are corrected to a standard of five insects.

Algal species	Duration of feeding							
	1 hour		4 hours		8 hours		21 hours	
	No. insects	Insect cpm; insect/fecal ratio below	No. insects	Insect cpm; insect/fecal ratio below	No. insects	Insect cpm; insect/fecal ratio below	No. insects	Insect cpm; insect/fecal ratio below
Diogenes bacillaris 30682	5	560 (3) 10	5	639 (3) 5	5	-- (53)*	2	4210 (2) 13
Lyngbya sp. 30718	5	549 (3) 2	5	4561 (.7) 19	2	8751 (.7) 36	4	10855 (.5) 70
Chlorococcum isabeliense 30912	5	1580 (2) 6	5	1919 (1) 4	1	2131 (7) 8	2	14341 (.7) 51
Chlorhormidium flaccidum 30927	5	1495 (2)	5	4150 (.7)	5	4683 (.7)	5	4564 (.7)

*Fecal counts per minute.

Table X. Algal carbon-14-assimilation elimination experiments, Method B. Numbers are corrected to a standard of three insects.

Algal species	Duration of excretion					
	1 hour		4 hours		8 hours	
	No. insects	Insect cpm; insect/fecal ratio below	No. insects	Insect cpm; insect/fecal ratio below	No. insects	Insect cpm; insect/fecal ratio below
Diogenes bacillaris	3	135 (4)	3	15 (7)	1	159 (5)
30682		2		.003		1
Chlorococcum isabeliense	3	245 (3)	3	501 (2)	3	28 (5)
30912		.8		--		.1
Chlorhormidium flaccidum	3	159 (4)	3	26 (5)	1	24 (2)
30927		.2		.03		.5

containing the sonicated algal cells and BBM, as well as a control plate with just BBM showed no signs of algal growth after 52 days. Algal cells were not observed on the insect body or legs upon direct examination of insects walking on algal colonies under 12X power or squashes of the insects using 43X power. See Appendix C for further results and discussion of algal utilization.

It was determined that five unlabelled insects had 10.4 $\pm$ 0 cpm of radioactivity which can be taken to be background or quenching, and indicates that they possessed no "natural" radioactivity.

Extrapolation to Nature

In assessing the ingestion and evacuation rates of algal material, it was observed that a minimum time of 15 minutes was needed to obtain green throughout the length of the insect, while 30 minutes was required for evacuation of that material. The cross-section of an algae-filled Onychiurus folsomii gut obtained with the use of a freezing microtome was observed to be cylindrical. Photographs of the cross section were not successful; and the sections were not always cylindrical being distorted by a dull razor blade, careless transference to the microscope slide or other reasons. Observed average cell diameters and calculated cell volumes of some algal species appear in Table XI. Empirically there is no correlation between algal cell volume, ingestion and growth indices, nor morphological habit, cell wall structure, such as the presence of a sheath, or population growth indices.

Table XI. Morphological habits, cell diameter and calculated cell volumes of algal species used in feeding experiments. Stock culture numbers accompany the algal species.

Algal species	Cell diameter (microns)	Cell volume (ml)	Morphological habit
Chlorhormidium flaccidum 29976	7.7	2.0×10^{-10}	Filamentous
Chlorococcum isabeliense 29967	13.3	1.2×10^{-9}	Solitary, coccoid
Chlorococcum minutum 30414	9.0	3.8×10^{-10}	Solitary, coccoid
Diogenes bacillaris 30450	2.9	3.1×10^{-11}	Solitary, coccoid
Diogenes sp. 29168	3.3	7.0×10^{-11}	Solitary, coccoid
Stichococcus sp. 30410	10.7	3.1×10^{-10}	Rod-shaped
Tetracystis aplanosporum 30501	12.6	1.0×10^{-9}	Colonial, coccoid
Anabaena sp. 30433	2.9	2.2×10^{-11}	Filamentous, sheath
Nostoc sp. 29303	2.5	1.4×10^{-11}	Filamentous, sheath
Oscillatoria sp. 30498	1.0	1.8×10^{-12}	Filamentous

DISCUSSION

Ingestion of algae is essential to the initial hypothesis that Onychiurus folsomii will grow on them. The hydrophobic nature of the exoskeleton would seem to preclude the movement of algal products into the insect, e.g. through the carapace by diffusion. After introduction to an algal plate a green "rod" soon became visible in an insect. Insects containing such green rods, presumably ingested algae, were carefully squashed and both fragments and whole algal cells were observed in the gut contents using a microscope, so there is no question but what Onychiurus will ingest at least 15 species of algae. These observations of algal cells in the guts of Onychiurus suggest that the ingested algae do play some role in nutrition.

Some of the algal cells from the gut and fecal matter grew in culture despite having passed through the gut. The resulting colonies on the plates were available to the insects and, being like the originally ingested matter, could supply a continuing food source. The results support Christiansen's suggestion that food particles are in fact recycled with nutriment being obtained during each passage through the insect's gut. It is possible however that Christiansen did not have a viable and reproducible algal cell in mind, but instead detrital particles which could be successively broken down. If an algal cell is viable when excreted as part of a fecal pellet in nature it can reproduce, thus keeping the algal population going and supplying a continuing food source. The hypothesis that algal materials are actually utilized will be considered in its place.

The question now is asked if the insects ingest algae in nature. Two kinds of evidence are found. First, not observing algae either in the guts, fecal material or squashes of field insects is not particularly disturbing. There are numerous other food materials present in the soil habitat such as fungi, bacteria and leaf detritus which to the insects may be more physically as well as numerically available, more efficiently obtained or more palatable than algae. This lack of evidence for ingestion of algae in the field, rather than being an apparent refutation of the initial hypothesis, merely suggests alternative hypotheses. It should be reiterated that no algae appeared on the agar plates used to maintain the insects, which contained soil particles and insect excreta.

Secondly, Figures 4 and 5 show that there is a co-occurrence of algae and Collembola found at different sites. This can be interpreted in two ways. First, this could mean that algae are in some way antagonistic to Collembola, reducing the Collembola presence when algal numbers are high. It was also observed that if the Collembolan population was large, few if any other insect species appeared on the collection plate. Secondly, there could be a positive food relationship, or importantly, some other mutual requirement, such that the algae are reduced as Collembola feed, and regain in numbers when the Collembolan population is reduced.

The third major hypothesis is that the ingested algae are utilized. Among the most gratifying results of this study has been the consistent success obtained in rearing Onychiurus folsomii as evidenced by its survival, increase in numbers and reproduction when

fed diets of various algae growing on the BBM agar plates. It is possible that some of the insects' success is due to the eating of fungi which are sometimes present on the plates, however no work was done with the fungi. The small size of the plates themselves on which the growth studies were conducted in near 100% humidity were found to have several advantages. As described, they were small enough to permit condensation on the inner surface of the top lid, which in dropping back onto the plate surface repeatedly re-wet the agar. In addition several were able to fit into the lypholyzing flask which enabled consistent and uniform carbon-14 labelling.

Several species of algae supported growth and reproduction of Onychiurus folsomii on agar plates while some algae and the media used did not sustain them. Growth of individuals is interpreted from the moulting observed but more accurately indicated by increases in the numbers of individuals. Since no correction factor could be made for the added number of insects to the plates, the population growth and moulting indices serve merely as indications of growth and reproduction of the Onychiurus population on the various plates. Though moulting of these insects may occur without an increase in length, growth is obviously dependent on the satisfaction of the nutritional requirements of the adult and larval stages which led eventually to reproduction.

The presence of three insect generations on several plates also lends credence to the hypothesis that algal products are assimilated. The data in Table X indicating time for insects to hatch as well as number hatched are similar to those obtained by Thibuad from other

Collembola species. Combining the results of various algal species, if fewer insects (4-5) are present on the plates, eggs are noted after 27 to 30 days. The average hatching time is between 10 and 30 days (Table VI) with the norm very close to 14 days. It should be noted that hatching time could be shorter than 14 days, because 12 days was the time interval used to observe many of the plates.

It appears from the results of the agar, BBM agar and silica gel experiments that Onychiurus folsomii can live, grow and reproduce on agar alone, as evidenced by the presence of eggs on the plain agar plates. The extent of nutriment provided by the BBM and agar is unknown. At the conclusion of several experiments however, it became certain the insects could utilize the agar, for they survived on it at least 85 days in contrast to a somewhat shorter time when BBM was added and 12 days for 84% of the insects on sterile silica gel plates. Some of the reduction in numbers on some plates is due to the mite predation but the significance of this has not been appraised. Although it was shown that at least on one agar plate the insect population could increase, this was not the case on the BBM agar. Pitting of the agar, indicating feeding, was observed on a plate containing eggs. In contrast however there was very little pitting observed on the algal feeding plates. Since the algae were grown on plates of BBM agar the utilization of that material does not seem to be an important factor in assessing the population growth and reproduction results of the insects fed on varieties of algal species.

Through the radioactive tracer experiments it was found that the cell products of some algal species were utilized by Onychiurus

folsomii and assimilated into the insect body in varying amounts. It is also concluded that algal contamination on the insect carapace does not contribute significantly to any radioactivity emitted. Radioactivity found in the insect tissues and its general increase with exposure time would indicate the utilization of part of the ingested labelled cells or cell byproducts. The hydrophobic nature of the exoskeleton, preventing labelled algal material from entering the insect except through its mouth opening, should be kept in mind.

Labelled cell byproducts are the most likely explanation for radioactivity found in the insects when fed algal species that also showed growth from fecal material, such as Chlorhormidium flaccidum or Diogenes bacillaris. The less than optimum environment for the algae in the insect gut could readily allow leaching of some cell product which would then be assimilated into the insect's tissues and utilized in its survival and growth. The algal cell would then be excreted in a viable condition as part of the fecal material, "recover" from the effects of the enzymes in the gut, and again reproduce when exposed to a suitable medium. Based on the radioactivity counts it is possible to speculate that only cell byproducts of C. flaccidum and D. bacillaris are used by the insect as the counts are considerably fewer than Chlorococcum isabeliense-fed insects. This alga also showed no algal growth from fecal material indicating that the entire cells are destroyed and utilized and no longer viable when excreted. Thus the radioactivity found in the tissues of empty-gutted insects after evacuation of radioactive algal cells, indicates not only ingestion of the algae but assimilation and implies incorporation of their substances into the insect's tissues.

While Christiansen specifically includes only unicellular algae as a possible diet, the present results have broadened the concept by showing that filamentous algal forms may also be utilized. The differences observed in relation to ingestion or growth do not seem to be correlated with the morphological characteristics of the algal species, such as cell shape, volume or filamentous structure nor with cell wall structure. However, different algal species produced different insect growth rates and reproductive success.

Using the average cell volume of Diogenes bacillaris and calculating the volume of the "average" Onychiurus folsomii gut as $2.4 \times 10^{-3} cm^3$, the maximum number of cells in the gut would be 7.7×10^7. Looking at Figures 5 and 6 the number of algal cells found using the methods described would not support the Collembolan population. However direct measurements such as the metabolism of the insects, the efficiency with which the insects use algae as a food, algal packing densities in the insect gut, or the size of the insect population present in the soil have not been made. It should also be remembered that these algal numbers are colonies per unit area, not volumetric. Likewise from appraisal of the techniques used it could well be that algal populations were significantly over- or underestimated. Keeping these factors in mind and assuming they offset one another it is suggested that the algal numbers obtained in the field are representative of actively growing algal colonies which are available to and may be used by O. folsomii. Based on the assimilation of algal products and subsequent growth it is safe to say then that algae may play a role in nutrition. It can also be said with some certainty that some of these

algal species, all of which were found in the same environment as Onychiurus folsomii are capable of providing the nutritional requirements and enough energy for the production, fertilization and hatching of eggs in addition to the maintenance of the adult's metabolism. Also these algae provide for the growth of both the adult and larval stages necessary to continue the population.

While there are many other interesting results as well as a few weaknesses in the above conclusions, the basic point to emanate from this study is that Onychiurus folsomii can live, grow and reproduce on individual algal species and may do so in nature.

APPENDIX A

The Soil Algal Population

The numbers of algal colonies per square centimeter of soil surface obtained are possibly misleading. Each colony on the agar plates was assumed to have arisen from a single cell or single "colony-forming" unit and the soil algal content was calculated on this assumption. Therefore an actual underestimation of the algal population may have been made because two or more cells or colony-forming units might remain together during sonication or even clump when pipetted onto the plate. Likewise, overestimation of field algal density could very well have been made. A single algal cell in the soil may not be on such a suitable substrate as the BBM growth medium which is available with all the required nutrients in a readily available form. There is also no way of knowing the percentage of spores actually present in the soil samples, thus constituting a non-growing and possibly non-available element to the insect in the field. It also is not known how many cells are present together in the soil, i.e. do they form a colony, and how many cells are needed to form a colony which is visible or attractable to the insect. It is not known if O. folsomii can recognize and ingest a single algal cell as might be commonly present in the soil, or if algal spores could be ingested and utilized.

As indicated by Figure 13 many algal colonies were found at depths to 9 cm, seemingly below a light level sufficient to sustain an algal population. It is possible that these cells were in fact spores which upon sonication and inoculation onto a nutrient

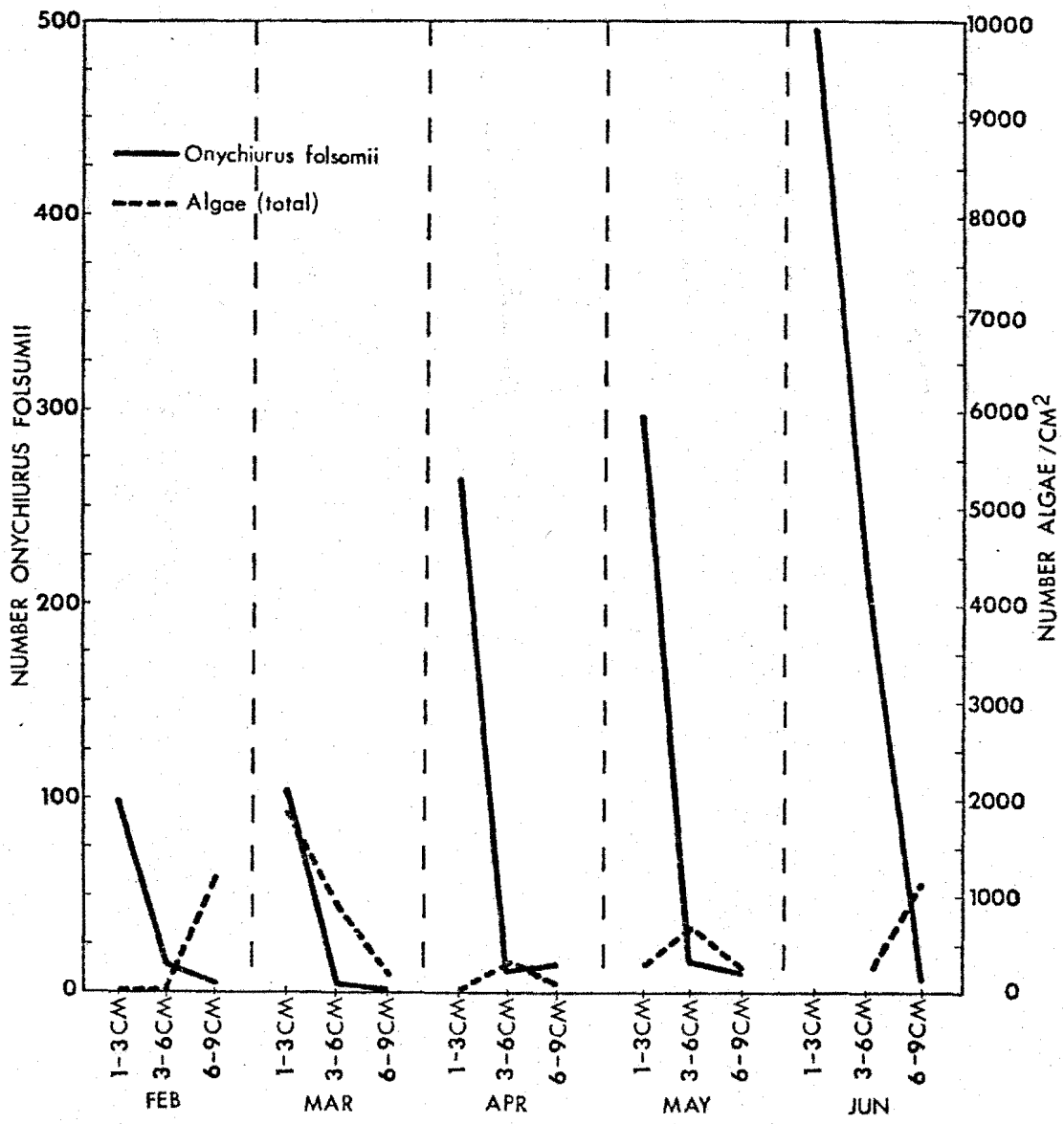

Fig. 13. Algal and Onychiurus folsomii collections from February through June, 1973. Three soil core depths, 1-3 cm, 3-6 cm and 6-9 cm were taken.

substratum started growing; or possibly cells that had been washed down via rain, transported from the soil surface by insect carapaces, or soil mixing by pigs, goats, etc. While there is no field evidence to support this, it is possible that if the insects were to ingest algal cells at the surface of the soil and walk down to 9 cm, that the algae found there result from defecated material of the insects. Some algal cells defecated from the insects in the laboratory are still viable, and if this in fact occurs in nature, could constitute a means of dispersal, other than rain wash, down through the soil. If subsequently it is found that Onychiurus does not contribute to the dispersal and distribution of the algal population, it is likely that some other soil arthropod does.

While it is commonly recognized that soil moisture is a very important factor in the distribution and survival of the Collembolan population, the quantitative importance of this effect has not been determined for algae. However, a mutual relationship between the algal and Collembolan populations which limits their distribution, can not be disregarded.

APPENDIX B

A Carbon-14 Labelling Method for Algae

The first method, Method A, devised to introduce labelled carbon dioxide provided insufficient and variable labelling of the algae. The method involved using the closed, lock-top petri dish as a self-contained unit. The needle point of a syringe was heated and a hole was carefully poked in the middle of the plastic petri dish lid with the hot needle point. The needle was left fixed in the petri dish lid as the plastic cooled. The plunger of the syringe had been removed and the barrel shortened. A rubber cap was placed on the end of the open syringe tube through which an intact needle and syringe could be poked to remove or add gas from the sealed petri dish. To seal the dish, white glue and a combination of paraffin and silicone sealers were used around the hole in the lid surrounding the needle, the top and bottom portions of the petri dish and the junction of the needle cap and syringe barrel. Using a syringe a volume of gas from the dish was removed to create a partial vacuum. Generated carbon-14 gas was added to each sealed petri dish. Ten Onychiurus folsomii were added to each of two labelled plates of Chlorococcum isabeliense, 30667 and 30804. As soon as the guts were completely green the insects were removed to a sterile BBM agar plate. After elimination of the guts, the fecal material and insects were put in separate counting vials and treated as described in the text. There was little labelling of the algae using this method, indicating a leak was present. Culture 30607 was apparently not assimilated by the insects, as both insect and fecal counts appear to be background.

Culture 30804 showed clearly that the insects assimilated some labelled algal material while label remaining in the fecal material was slight. Due to the seeming inconsistencies involved with the labelling method, it was abandoned.

Some of the C^{14} samples were "lost" due to insects escaping from the covered petri dishes, miscounting, insects not having observably ingested any algal material, or otherwise disappearing via some "possibly ethereal" source. The variability of the radioactive tracing results could also result from leakage or the variability of inoculation, possibly affecting the uptake by algal cells. The variable results of the C^{14}-assimilation results could also reflect individual preferences of the insect evidenced by differential feeding, though this was avoided to some extent by using only those insects with full guts to eliminate and then count radioactive emissions. According to Healey, in feeding fungi to Onychiurus procampatus, the amount of food assimilated varies widely between species of fungi as well as between different cultures of the same fungus.

As previously mentioned it was observed in photographs and under the microscope that algal cell walls are sometimes broken down in the gut of O. folsomii. There is not a distinguishable difference in the ingested cell structure between foregut, midgut and hindgut. The midgut in many insects is the site for absorption of digested material. Folsom and Welles (1906) suggest that the midgut functions significantly in excretion as evidenced by the casting off of the midgut epithelium prior to moulting. Since it was not known in

what state of moulting the experimental insects were, this could greatly affect the assimilation of algal material and produce results incompatible with the algal feeding experiments.

APPENDIX C

Reasons for Variability of Results

Several points should be brought out as to the possible reasons for the variability of results of the foregoing experiments. Initially, it should be recognized that O. folsomii individuals were very limited in numbers, even despite attempts to find an additional source on Oahu. Several cores were made at various points around the island of Oahu and no Collembola were extracted.

As for the feeding experiments, pressures encountered in nature are lacking on an agar plate in the laboratory. There was a constant and abundant food source available without competition from predators. Overcrowding was possibly a factor not encountered to such a degree in nature but that this was adverse was not observed from the growth results.

The insects are also accustomed to darkened conditions in their natural soil habitats. Insects put on sterile plates under incubation lights usually died within 3 days probably due to high light intensity. The increased amount and intensity of light could possibly have altered the physiological reactions of the insects and subsequently the results of various experiments.

Concerning the insect population, the numbers of males and females were unknown and if one was exceedingly dominant, the lack of increase in numbers of insects as well as the absence of eggs would be evident on the algal plates. In analysis of the results it was assumed that the proportion of male to female insects used for each experiment was 1:1, presumably the same encountered in nature. The

ages of the insects are also unknown and older insects could possibly be clumped on a single plate, producing mortality not directly linked to the algal species as food. Again it was assumed that age was constant in all of the experiments.

Results of the feeding experiments also could be questioned on the basis of stomach contents of the insects when added to the various experimental plates. If fungal and/or bacterial populations existed in the gut, and were excreted, these could possibly reproduce on the BBM agar. Undigested spores passed through the gut are another source of contamination and a very possible food source. Differences in the results between replicate plates could also be attributed to the general "health" of the cultures. Although attempts were made to insure uniform spraying of the culture plates, differences in numbers of cells to hit the agar surface, and live to reproduce, the agar surface tension and humidity, and bacterial and fungal population in the spray as well as the culture could all be contributing factors to varying results.

Fungal and bacterial contamination, whether initiating from the insect gut, or through contamination in the air, plays an undefined role. If in fact fungal material was present on the labelled algal plates, and the insect ingested both labelled fungi and algae, and assimilated the fungi, but passed the algal cells unassimilated, then the C^{14} results are meaningless. On a longer term basis, such as the feeding experiments, fungi could possibly have supported the insects on some of the plates, or perhaps those where corroborative evidence from assimilation experiments was not

present, but this is highly unlikely due to common observation of algae in the insect gut and evidence to support assimilation of algal material into the insect tissues.

It should be noted that the carapaces of a few of the insects on agar plates started turning brown, a few while alive and a few when dead. The dark brown color started at one end of the body and gradually most of the carapace would be markedly discolored. This occurred on agar plates containing algae, as well as "sterile" agar plates.

In hopes of obtaining algal ingestion and assimilation data that could be statistically analyzed, gravimetric measurements were tried but soon abandoned. It was attempted to weigh the insects before and after feeding and to weigh the fecal material, but this proved very difficult due to the delicacy of the fecal material, the small number of O. folsomii individuals, and the sensitivity of the instrument.

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